Kidney International, Vol. 65 (2004), pp. 1647–1653

Pioglitazone increases renal tubular cell albumin uptake but limits proinflammatory and fibrotic responses

STEPHEN ZAFIRIOU, SCOTT R. STANNERS, TANIA S. POLHILL, PHILIP PORONNIK, and CAROL A. POLLOCK

Department of Medicine, University of Sydney, Kolling Institute of Medical Research, Royal North Shore Hospital, St. Leonards, New South Wales, Australia; and School of Biomedical Sciences, University of Queensland, St. Lucia, Queensland, Australia

Pioglitazone increases renal tubular cell albumin uptake but limits proinflammatory and fibrotic responses.

Background. Peroxisome proliferator-activated receptor gamma (PPARγ) agonists, which are known to be critical factors in lipid metabolism, have also been reported to reduce proteinuria. The mechanism and its relevance to progressive nephropathy have not been determined. The aims of this study were to assess the direct effects of a PPARγ agonist on tubular cell albumin uptake, proinflammatory and profibrotic markers of renal pathology, using an opossum kidney model of proximal tubular cells.

Methods. Cells were exposed to pioglitazone (10 μmol/L) in the presence and absence of low-density lipoprotein (LDL) 100 μg/mL ± exposure to albumin 1 mg/mL. Results were expressed relative to control (5 mmol/L glucose) conditions.

Results. Pioglitazone caused a dose-dependent increase in tubular cell albumin uptake ($P < 0.0001$). Despite the increase in albumin reabsorption, no concurrent increase in inflammatory or profibrotic markers were observed. Exposure to LDL increased monocyte chemoattractant protein-1 (MCP-1) ($P < 0.05$) and transforming growth factor-β1 (TGF-β1) ($P < 0.05$) production, which were reversed in the presence of pioglitazone. LDL induced increases in MCP-1 and TGF-β1 were independent of nuclear factor-κB (NF-κB) transcriptional activity. In contrast, tubular exposure to albumin increased tubular protein uptake, in parallel with an increase in MCP-1 ($P = 0.05$), TGF-β1 ($P < 0.02$) and NF-κB transcriptional activity ($P < 0.05$), which were unaffected by concurrent exposure to pioglitazone.

Conclusion. These findings suggest that dyslipidemia potentiates renal pathology through mechanisms that may be modified by PPARγ activation independent of NF-κB transcriptional activity. In contrast, tubular exposure to protein induces renal damage through NF-κB–dependent mechanisms that are unaffected by PPARγ activation.

Key words: proximal tubule cell, peroxisome proliferator-activated receptor, albumin, monocyte chemo-attractant protein-1, transforming growth factor-β1.

Received for publication October 17, 2003
Accepted for publication December 12, 2003

Proteinuria is predictive of progressive deterioration in renal function in patients with various forms of renal disease, including diabetes mellitus and primary glomerular pathology [1–5]. The proinflammatory and profibrotic effects of albumin on the proximal tubular cell are well recognized [6, 7]. More recently, the presence of lipid abnormalities, including elevated levels of native low-density lipoproteins (LDL), have been implicated both in the accelerated decline in renal function and in the increased vascular complications observed in patients with renal disease [8, 9]. Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of ligand-dependent transcription factors and have been shown to be critical factors in lipid metabolism [10]. It is clear that the thiazolodinedione PPAR gamma (PPARγ) ligands additionally reduce albuminuria in both humans with type II diabetes mellitus and in animal models [11–13], but the relevance of this to progressive renal disease has not been determined. In particular, the effect of stimulating PPARγ activity on objective markers of tubulointerstitial renal disease, which correlate most highly with functional renal decline, is unknown. PPARγ activation has been shown to modify the proatherogenic cytokine production inherent in atherogenesis, such as enhanced interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α) [14, 15], and glomerular production of transforming growth factor-β1 (TGF-β1) [16, 17], but studies on the effects of PPARγ in the tubulointerstitium are lacking. PPARγ agonists have previously been considered to inhibit the activity of proinflammatory transcription factors such as nuclear factor-κB (NF-κB), activating protein-1 (AP-1), and signal transducer and activator of transcription (STAT) [18]. However, more recently it is recognized that mediators independent of transcriptional factors such as NF-κB play a role in the complications of diabetes mellitus, including nephropathy [19].

Current evidence would suggest that PPARγ expression in the kidney predominates in renal medullary collecting ducts with lower levels in glomeruli and

microvasculature [20, 21]. However, the proximal tubule accounts for 90% of the volume of the kidney, tubulointerstitial disease predicts declining renal function and thickening of the proximal tubular basement membrane is the earliest manifestation of nephropathy in patients with diabetes mellitus. Hence, the current investigation aimed to assess the direct effects of a PPARγ agonist on tubular cell albumin uptake, proinflammatory and profibrotic markers of renal pathology following exposure to LDL in the presence or absence of albumin.

METHODS

Cell culture

Opossum kidney cells were used as an invitro model of proximal tubular cells. Cells were routinely grown in plastic tissue culture flasks (75 cm^2) in Dulbecco's modified Eagle's media and Hams F-12 (DMEM/F-12) (ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA), supplemented with 10% fetal calf serum (FCS) (Trace Biochemicals, Sydney, Australia), 2 mmol/L L-glutamine (catalogue number 17-605E) (Bio-Whittaker, Walkersville, MD, USA), and 50 U/mL penicillin and 50 μg/mL streptomycin (catalogue number 17-603E) (Bio-Whittaker). Cells were grown to confluence in a 95% air-5% CO_2 incubator.

Isolation of LDL

LDL was prepared as previously described [22]. Briefly, whole blood obtained from individual normolipidemic healthy donors was collected into 50 mL tubes containing disodium ethylenediaminetetraacetate (EDTA) (200 mmol/L, 0.5 mL), aprotinin (5 to 10 trypsin inhibitory units, 50 μL (Sigma-Aldrich, St. Louis, MO, USA), PPACK (1 μmol/L final concentration, D-phenylalanyl-L-arginine chloromethyl ketone) (Calbiochem, San Diego, CA, USA) and soybean trypsin inhibitor (0.2 mg/mL final concentration) (Sigma-Aldrich). Plasma was separated by centrifugation (3000 rpm (16000*g*), 20 minutes, 10°C). LDL (1.05 > density > 1.02 g/mL) was isolated on a discontinuous gradient using a Beckman L8-M ultracentrifuge (Palo Alto, CA, USA) and a VTi50 rotor (206,000*g* (average), 2.5 hours, 10°C). A second centrifugation at a density of 1.063 g/mL using a Ti70 rotor (184,000*g* (average), 22 hours, 10°C) removed traces of contaminating albumin. The LDL was dialysed against four exchanges of deaerated Dulbecco's modified phosphate-buffered saline (PBS), pH 7.4 (Sigma-Aldrich), containing chloramphenicol (0.1 g/L) and EDTA (1 g/L), then filter-sterilized (0.45 μm). LDL was then stored in the dark at 4°C and used within 14 days, at which time the LDL was desalted by passing it through two Sephadex G-25 M columns (Column PD-10) (Amersham Pharmacia Biotech UK, Buckinghamshire, England) using PBS (treated with Chelex 100 resin) (Bio-Rad, Hercules, CA, USA) as the buffer. LDL protein concentration was determined using the Bio-Rad Protein Assay.

Experimental protocol

All experiments were carried out on quiescent, confluent opossum kidney cells. Cells were made quiescent by incubation for 24 hours in control media (DMEM/F-12 containing 5 mmol/L glucose and antibiotics as above) without serum. The cells were exposed for 24 hours to media containing (*1*) 5 mmol/L D-glucose alone (control) ± pioglitazone (initial dose finding studies were performed at concentrations ranging from 0.1 to 10 μmol/L pioglitazone and subsequent experiments using pioglitazone were undertaken using 10 μmol/L pioglitazone as no cytotoxicity was demonstrated and growth responses had plateaued from those observed using 3 μmol/L pioglitazone); (*2*) 100 μg/mL LDL ± 10 μmol/L pioglitazone (this concentration of LDL was shown in dose-finding studies to have no cytotoxicity, maximal pathophysiologic effects, and is a clinically relevant concentration of LDL); (*3*) 1 mg/mL albumin ± 10 μmol/L pioglitazone (this concentration was selected to mimic the concentration of albumin that the proximal tubule would be exposed to in proteinuric states); and (*4*) 1 mg/mL albumin and 100μg/mL LDL ± 10 μmol/L pioglitazone.

After 24 hours, the cells were studied as detailed below.

PPARγ expression in opossum kidney cells

PPARγ protein expression in opossum kidney cells was confirmed by Western blot analysis. Cells were lysed in cell lysis buffer containing 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100, 20 mmol/L Tris, pH 7.5, and "Complete" protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Triton X-100 soluble fractions were collected following centrifugation of the lysed cells at 14,000 rpm for 10 minutes. Laemmli buffer was added and the samples separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes and then blocked overnight at 4°C in 5% skim milk in Tris-buffered saline (TBS)/Tween20 (Sigma Chemical, St. Louis, MO, USA). The membranes were incubated with mouse anti-PPARγ antibody (1:1000) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at room temperature for 2 hours and after washing with TBS/Tween 20, incubated for a further 60 minutes at room temperature with an antimouse IgG horseradish peroxidase-conjugated antibody (1:1600) (Amersham Biosciences UK, Little Chalfont, Buckinghamshire, England). The antibody used in this study recognizes both PPARγ isoforms 1 and 2. Bound antibodies were detected using enhanced chemiluminescence (ECL) Western Blot Kit (Amersham Biosciences UK) and visualized on chemiluminescence film (Hyperfilm ECL) (Amersham Pharmacia Biotech UK).

Assessment of cell growth

Cell counts, as a measure of cell proliferation, were performed manually on trypsinized cells using a hemocytometer in each of the experimental conditions. The total protein content of cells was determined as a marker of cellular hypertrophy. Cells were solubilized with 0.2 mol/L NaOH and the protein measured using a Bio-Rad protein assay.

Albumin uptake

Cells were grown in 48-well plates to confluence and were exposed to the experimental conditions outlined above. The cells were then washed three times with ice-cold Hepes-Ringer (pH 6.0). After incubation in Hepes-Ringer (pH 7.4) containing 100 μg/mL Texas Red–labeled bovine serum albumin (BSA) for 2 hours at 37°C, unbound Texas Red–BSA was removed by rinsing five times with ice-cold Hepes-Ringer (pH 7.4). Finally, the cells were lysed using 3[N-morpholino] propane sulfonic acid (MOPS) solution containing the detergent Triton X-100 (0.1% vol/vol), which ensured that all fluorescence measurements were performed at pH 7.4. The intracellular fluorescence was measured using a spectrofluorometer (Fusion, Packard, Perkin Elmer Life Sciences, Boston, MA, USA) at an excitation wavelength of 580 nm and an emission wavelength of 630 nm. Cells were solubilized with 0.2 mol/L NaOH and the protein content measured.

Cytokine production

Active TGF-β1 and monocyte chemoattractant protein-1 (MCP-1) production were measured on supernatants using commercially prepared enzyme-linked immunosorbent assay (ELISA) kits (TGF-β1 E_{max} ImmunoAssay System) (Promega Corporation, Madison, WI, USA and BioSource International, CA, USA).

NF-κB specific reporter gene assay

The activation of a NF-κB–specific cis-acting enhancer element was surveyed in vitro using the Mercury Pathway Profiling SEAP (secreted alkaline phosphatase) System (Clontech Laboratories, Inc., Palo Alto, CA, USA). The SEAP gene served as the target gene. At 24 hours after plating, the opossum kidney cells were 80% confluent and they were transiently transfected with the pNF-κB-SEAP using the Effectene Transfection Reagent (Qiagen, GmbH, Hilden, Germany). The transfection reagent was removed after 8 to 10 hours and fresh growth media applied. After a further 24 hours, the cells were quiesced in serum-free medium for 24 hours and then exposed to the defined experimental conditions for 24 hours. At this time the supernatants were collected and the cells were washed with PBS and then solubilized with 0.2 mol/L NaOH for determination of total protein.

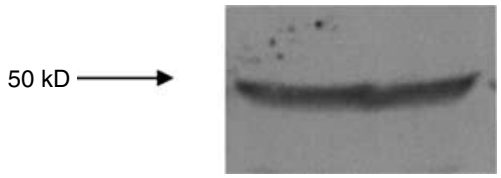

Fig. 1. Peroxisome proliferator-activated receptor gamma (PPARγ) is expressed in opossum kidney cells. Western Blot of opossum kidney cell lysate showing that PPARγ is expressed in opossum kidney cells and has a molecular weight of ~50 kD.

NF-κB–dependent SEAP reporter protein expression was measured in serum-free culture medium samples (1:4 diluted with SEAP buffer) after heat inactivation of endogenous alkaline phosphatase (incubation at 65°C for 30 minutes) using disodium 3-(4-methoxyspiro{1, 2-dioxetane-3, 2′-(5′-chloro)tricyclo[3, 3, 1, $1^{3,7}$] decan}-4-yl) phenyl phosphate (CSPD) Substrate (Applied Biosystems, Bedford, MA, USA) as a chemiluminescent substrate for SEAP and measuring the peak light emission using a luminometer (Fusion, Packard, Perkin-Elmer Life Sciences). Results were corrected for cell protein.

Statistical analysis

All experimental conditions were replicated three to eight times. All results are expressed as a percentage of the control value (100%). Results are expressed as mean ± SEM. Statistical comparisons between groups were made by analysis of variance (ANOVA) unless otherwise stated. Pairwise multiple comparisons were made by the Fisher protected least-significant differences test. Analyses were performed using the software package, StatView, version 5.01 (Abacus Concepts, Inc., Berkley, CA, USA). *P* values less than 0.05 were considered significant.

RESULTS

Detection of PPARγ in opossum kidney cells

Western blotting demonstrated a single band of molecular weight ~50 kD indicating that PPARγ1 is the predominant isoform in these cells (Fig. 1).

Cell growth (cell number and cell protein)

Hypertrophy was induced by exposure to LDL (114 ± 3%; $P < 0.0001$), albumin (127 ± 2%; $P < 0.0001$), and pioglitazone (119 ± 3%; $P < 0.0001$). Pioglitazone did not modify the hypertrophic effect of LDL or albumin. No treatment altered the cell proliferative response (Fig. 2).

Albumin uptake

Exposure of opossum kidney cells to pioglitazone resulted in a dose-dependent increase in albumin uptake, which was maximal at a concentration of 10 μmol/L, being

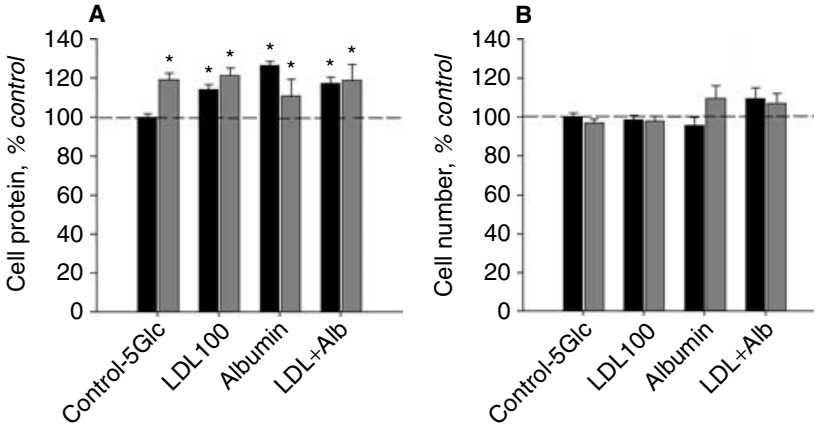

Fig. 2. Growth in opossum kidney cells exposed to pioglitazone with and without low-density lipoprotein (LDL) and/or albumin. Cell protein (*A*) and cell number (*B*) in opossum kidney cells exposed to the defined treatments for 24 hours. Symbols are: (■) 5 mmol/L glucose (5Glc); (▣) 5 mmol/L glucose with pioglitazone 10 μmol/L; LDL 100, LDL 100 μg/mL; albumin, albumin 1 mg/mL; LDL + Alb, LDL 100 μg/mL + albumin 1 mg/mL.

128 ± 5% of control values ($P < 0.0001$) (Fig. 3A). Hence this concentration was used in subsequent experiments. Exposure to LDL reduced albumin uptake to 78 ± 5% of control values ($P < 0.0001$) (Fig. 3B), in the absence of any observed cytotoxicity (data not shown), which was not modified in the presence of pioglitazone. Albumin uptake was stimulated by prior exposure to albumin to 116 ± 7% of control ($P = 0.05$), which was further enhanced in the concurrent presence of pioglitazone to 159 ± 12% of control values ($P < 0.0001$). Exposure to the combination of LDL and albumin did not alter tubular albumin uptake, and the addition of pioglitazone to the combination of LDL and albumin, had no observed effect.

TgF-β1 production

Pioglitazone 10 μmol/L alone had no significant effect on TGF-β1 production. Exposure of opossum kidney cells to LDL caused a significant increase in TGF-β1 production (140 ± 15%; $P < 0.05$). Pioglitazone reversed this effect (Fig. 4). Exposure to albumin also stimulated TGF-β1 production (126 ± 11%; $P < 0.02$). However, concurrent exposure to pioglitazone did not modify this response. The combination of LDL and albumin did not alter TGF-β1 production.

MCP-1 production

Pioglitazone 10 μmol/L alone had no significant effect on MCP-1 production. Exposure of opossum kidney cells to LDL 100 μg/mL caused a significant increase in MCP-1 production (185 ± 23%; $P < 0.05$), which was reversed with the addition of pioglitazone 10 μmol/L (Fig. 5). Albumin resulted in a significant increase in MCP-1 (289 ± 116%; $P = 0.05$), which was not reversed by pioglitazone. The combination of LDL and albumin did not alter MCP-1 production.

NF-κB reporter gene assay

Pioglitazone 10 μmol/L alone had no significant effect on NF-κB promoter activity. Exposure of opossum kidney cells to LDL 100 μg/mL in the absence or presence of albumin caused a significant decrease in NF-κB promoter activity (74 ± 3%; $P < 0.01$, and 76 ± 2%; $P < 0.01$ respectively). The addition of pioglitazone to LDL ± albumin did not modify this response (Fig. 6). Albumin exposure to opossum kidney cells stimulated NF-κB activity (123 ± 6%; $P < 0.05$) and this was not altered in the presence of pioglitazone.

DISCUSSION

In the present study we have confirmed the presence of the PPARγ isoform γ1 in the opossum kidney proximal tubule model. PPARγ2 is known to primarily occur in adipose tissue and PPARγ1 more ubiquitously expressed. However, in the kidney it has largely been previously described in the distal tubule of the kidney, with lesser expression in the vasculature and glomeruli [21]. The finding that PPARγ was expressed in the proximal tubule, which forms the bulk of the renal mass and is specifically implicated in the tubulointerstitial response to renal injury, characteristic of progressive nephropathy, suggested that the role of PPARγ agonists in proximal tubular cell models should be further explored.

The present study has demonstrated that the reduction in proteinuria observed following treatment with PPARγ agonists is at least partly explained by increased tubular cell albumin uptake. Of concern is that increased "protein trafficking" may have potential deleterious consequences. MCP-1 and additional cytokines such as IL-8 have been previously implicated in the proinflammatory consequence of enhanced tubular albumin uptake [23–25]. Our experiments clearly demonstrate that enhanced tubular uptake of albumin induced by prior exposure of cells to albumin results in increased production of the proinflammatory cytokine MCP-1 and the

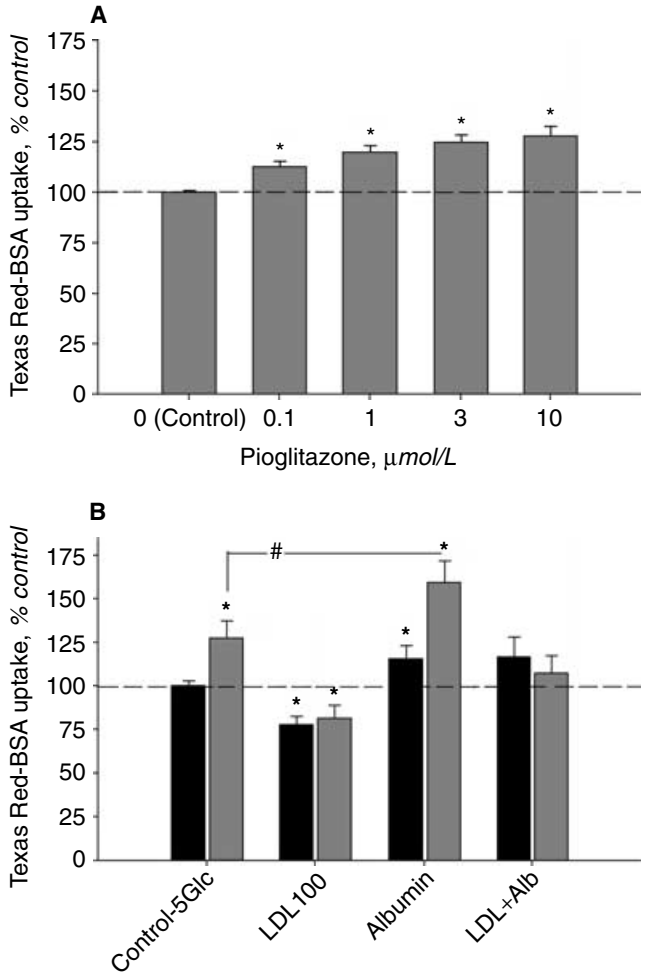

Fig. 3. Albumin uptake in opossum kidney cells. (*A*) Following 24 hours' exposure to different concentrations of pioglitazone (0.1 to 10 μmol/L), opossum kidney cells were incubated with Texas Red–bovine serum albumin (BSA) (100 μg/mL) for 2 hours, after which time the intracellular Texas Red–BSA uptake was measured and corrected for cell protein. Results are standardized against control conditions (opossum kidney cells in 5 mmol/L glucose). (*B*) Albumin uptake in opossum kidney cells exposed to pioglitazone with and without low-density lipoprotein (LDL) and/or albumin. Following 24 hours' exposure to the defined treatments, opossum kidney cells were incubated with Texas Red–BSA (100 μg/mL) for 2 hours, after which time the intracellular Texas Red–BSA uptake was measured and corrected for cell protein. Symbols are: (■) 5 mmol/L glucose (5Glc); (▣) 5 mmol/L glucose with pioglitazone 10 μmol/L; LDL 100, LDL 100 μg/mL; albumin, albumin 1 mg/mL; LDL + Alb, LDL 100 μg/mL + albumin 1 mg/mL.

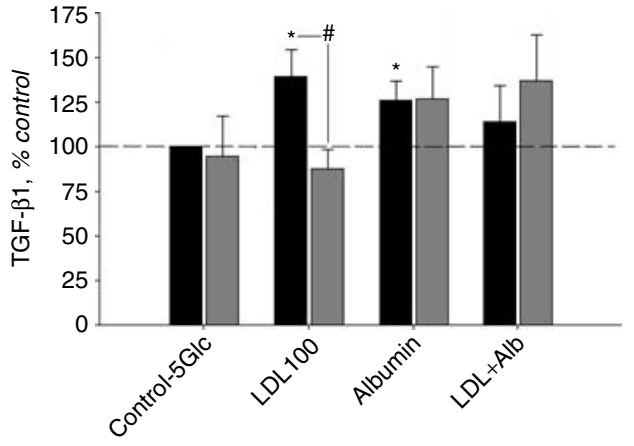

Fig. 4. Transforming growth factor-β1 (TGF-β1) secretion in opossum kidney cells exposed to pioglitazone with and without low-density lipoprotein (LDL) and/or albumin. Active TGF-β1 levels were measured by enzyme-linked immunosorbent assay (ELISA) in media conditioned by opossum kidney cells exposed to the defined experimental treatments for 24 hours. Symbols are: (■) 5 mmol/L glucose (5Glc); (▣) 5 mmol/L glucose with pioglitazone 10 μmol/L; LDL 100, LDL 100 μg/mL; albumin, albumin 1 mg/mL; LDL + Alb, LDL 100 μg/mL + albumin 1 mg/mL. $^{\#}P < 0.05$ for LDL 100 μg/mL vs. LDL 100 μg/mL with pioglitazone 10 μmol/L.

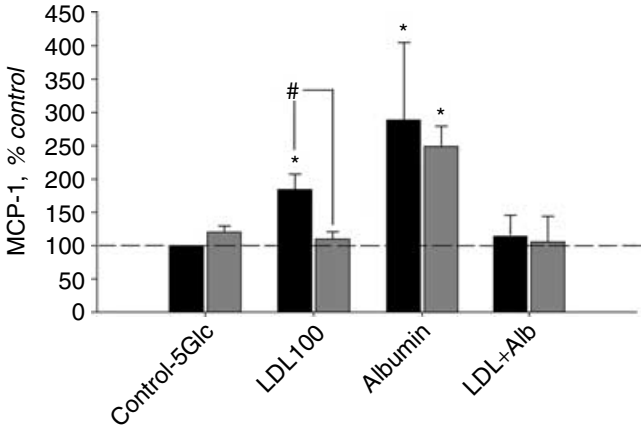

Fig. 5. Monocyte chemoattractant protein-1 (MCP-1) secretion in opossum kidney cells exposed to pioglitazone with and without low-density lipoprotein (LDL) and/or albumin. MCP-1 levels were measured by enzyme-linked immunosorbent assay (ELISA) in media conditioned by opossum kidney cells exposed to the defined experimental treatments for 24 hours. Symbols are: (■) 5 mmol/L glucose (5Glc); (▣) 5 mmol/L glucose with pioglitazone 10 μmol/L; LDL 100, LDL 100 μg/mL; albumin, albumin 1 mg/mL; LDL + Alb, LDL 100 μg/mL + albumin 1 mg/mL. $^{\#}P < 0.05$ for LDL 100 μg/mL vs. LDL 100 μg/mL with pioglitazone 10 μmol/L.

profibrotic cytokine TGF-β1. In contrast, the enhanced albumin uptake induced by pioglitazone was not associated with an increased inflammatory or profibrotic cytokine response. Hence, proteinuria is reduced without potential deleterious effects on the tubulointerstitium. We have recently demonstrated that tubular albumin uptake is associated with increased activity of the sodium-hydrogen exchanger [26], responsible for sodium reabsorption in the proximal tubule. Although not addressed in the present study, it is interesting to speculate that the edema observed with the thiazolidinediones [27] is due to enhanced tubular sodium reabsorption inherent in the increase in tubular albumin uptake.

The presence of lipid abnormalities, including elevated levels of native LDL, have been implicated in an accelerated decline in renal function [8], which is supported mechanistically by the findings of the present study. As PPAR agonists have been shown to be critical factors in

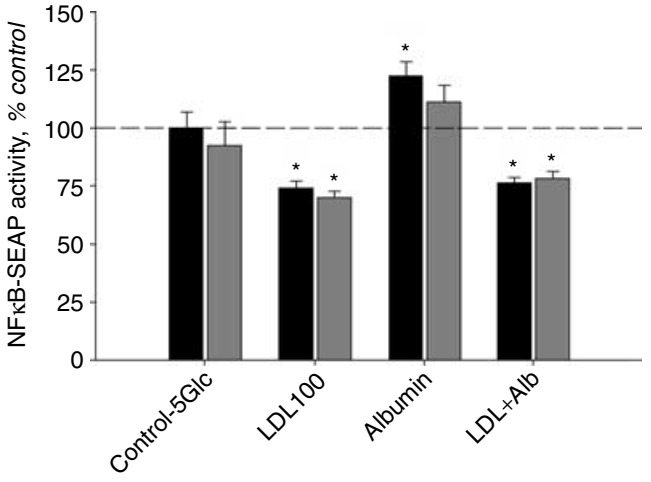

Fig. 6. Nuclear factor-κB (NFκB)-secreted alkaline phosphatase (SEAP) activity in opossum kidney cells exposed to pioglitazone with and without low-density lipoprotein (LDL) and/or albumin. NF-κB–specific reporter gene assay. SEAP expression of opossum kidney cells exposed to the defined treatments for 24 hours. Symbols are: (■) 5 mmol/L glucose (5Glc); (□) 5 mmol/L glucose with pioglitazone 10 μmol/L; LDL 100, LDL 100 μg/mL; albumin, albumin 1 mg/mL; LDL + Alb, LDL 100 μg/mL + albumin 1 mg/mL.

lipid metabolism, we addressed the effect of pioglitazone on the tubular cell responses to LDL. Our results confirm that dyslipidemia may be a key factor in the development of progressive renal disease. Specifically, exposure of opossum kidney cells to LDL increased MCP-1 and TGF-β1 production, which were reversed in the presence of pioglitazone. Recently, it has been shown in renal and nonrenal tissue that PPARγ agonists increase cholesterol efflux from cells due to induction of the cholesterol efflux pump ABC-A1 [28]. Reduced cellular accumulation of cholesterol may therefore be a mechanism whereby the specific deleterious effects of LDL are ameliorated in the kidney. Interestingly, despite the independent deleterious effects of LDL and albumin on the tubular cells, the present study did not demonstrate that the combination of LDL and albumin induced any change in cytokine release. It is likely that the lipid associated with albumin and was therefore not taken up into the tubular cells to induce pathology. Clearly, the concurrent exposure of albumin and LDL reduced albumin uptake and hence tubular pathology. However, the mechanism whereby this occurs is not known.

Although PPARγ agonist activity has been previously attributed to inhibition of the action of nuclear transcription factor NF-κB, STAT and AP-1 have also been implicated [14, 15, 29]. Our results suggest that in contrast to the induction of NF-κB transcriptional activity by exposure to albumin, LDL induced increases in MCP-1 and TGF-β1 were independent of NF-κB transcriptional activity. Indeed, the proinflammatory and profibrotic responses induced by exposure to albumin were not modified by concurrent exposure to pioglitazone, suggesting that pioglitazone is acting through pathways other than NF-κB.

The transcriptional regulation of MCP-1 has previously been attributed, at least in part to NF-κB dependent pathways [24]. However, the promoter regions that control MCP-1 gene expression are variable and suggest extensive interactions between both transcription factors and promoter elements [30]. The murine MCP-1 promoter contains AP-1 and specificity protein-1 (SP-1), in addition to NF-κB promoter sites, hypermethylation and orphan sites, all of which regulate MCP-1 activity [30]. In the opossum kidney cell model and in humans, the signaling pathways that govern MCP-1 expression are not known. NF-κB–independent pathways have been observed in adipocytes where the thiazolidinedione, troglitazone, was shown to have no effect on NF-κB activation and DNA binding activity in response to TNF-α. Clearly, modification of NF-κB by factors such as IκB-α may still mediate an effect of NF-κB independent of transcriptional activity. However, to date this has not been addressed. The reduction in TGF-β1 secretion induced by pioglitazone in the presence of LDL suggests that the profibrotic effects of LDL may also be abrogated by PPARγ agonists. As TGF-β1 is regulated through an AP-1 promoter this also suggests that pioglitazone is acting through NF-κB–independent mechanisms. TGF-β1 is clearly implicated in all forms of progressive renal disease. Hence, its modification is of key therapeutic significance.

CONCLUSION

We have demonstrated the key role that elevated LDL levels may play in the development of tubular pathology characteristic of progressive renal disease. Tubular dysfunction mediated by exposure to LDL occurs in association with increased secretion of the proinflammatory and profibrotic cytokines MCP-1 and TGF-β1, which are normalized in the presence of the PPARγ agonist pioglitazone. Our findings suggest that dyslipidemia potentiates renal pathology through mechanisms independent of NF-κB transcriptional activity. In contrast, tubular exposure to protein induces renal damage through NF-κB–dependent mechanisms that are unaffected by PPARγ activation. These findings suggest that PPARγ agonist therapy in patients with metabolic characteristics inherent in renal disease may reduce proteinuria and potentially delay progression of tubulointerstitial disease by reducing the inflammatory and fibrotic response.

ACKNOWLEDGMENTS

These studies were supported by the National Health and Medical Research Council of Australia and the Juvenile Diabetes Research Foundation.

Reprint requests to Professor Carol A. Pollock, Department of Medicine, Level 3, Wallace Freeborn Bldg., Royal North Shore Hospital, St. Leonards, Sydney, NSW 2065, Australia.
E-mail: carpol@med.usyd.edu.au

REFERENCES

1. Wapstra FH, Navis G, de Jong PE, de Zeeuw D: Prognostic value of the short-term antiproteinuric response to ACE inhibition for prediction of GFR decline in patients with nondiabetic renal disease. *Exp Nephrol* 4 (Suppl 1):47–52, 1996
2. Breyer JA, Bain RP, Evans JK, *et al*: Predictors of the progression of renal insufficiency in patients with insulin-dependent diabetes and overt diabetic nephropathy. The Collaborative Study Group. *Kidney Int* 50:1651–1658, 1996
3. Peterson JC, Adler S, Burkart JM, *et al*: Blood pressure control, proteinuria, and the progression of renal disease. The Modification of Diet in Renal Disease Study. *Ann Intern Med* 123:754–762, 1995
4. Ruggenenti P, Perna A, Mosconi L, *et al*: Urinary protein excretion rate is the best independent predictor of ESRF in non-diabetic proteinuric chronic nephropathies. "Gruppo Italiano di Studi Epidemiologici in Nefrologia" (GISEN). *Kidney Int* 53:1209–1216, 1998
5. Jafar TH, Stark PC, Schmid CH, *et al*: Proteinuria as a modifiable risk factor for the progression of non-diabetic renal disease. *Kidney Int* 60:1131–1140, 2001
6. Zoja C, Morigi M, Remuzzi G: Proteinuria and phenotypic change of proximal tubular cells. *J Am Soc Nephrol* 14:S36–S41, 2003
7. Donadelli R, Abbate M, Zanchi C, *et al*: Protein traffic activates NF-kB gene signaling and promotes MCP-1-dependent interstitial inflammation. *Am J Kidney Dis* 36:1226–1241, 2000
8. Dominguez JH, Tang N, Xu W, *et al*: Studies of renal injury. III. Lipid-induced nephropathy in type II diabetes. *Kidney Int* 57:92–104, 2000
9. Fried LF, Orchard TJ, Kasiske BL: Effect of lipid reduction on the progression of renal disease: A meta-analysis. *Kidney Int* 59:260–269, 2001
10. Guan Y: Targeting peroxisome proliferator-activated receptors (PPARs) in kidney and urologic disease. *Minerva Urol Nefrol* 54:65–79, 2002
11. Fujii M, Takemura R, Yamaguchi M, *et al*: Troglitazone (CS-045) ameliorates albuminuria in streptozotocin-induced diabetic rats. *Metabolism* 46:981–983, 1997
12. Imano E, Kanda T, Nakatani Y, *et al*: Effect of troglitazone on microalbuminuria in patients with incipient diabetic nephropathy. *Diabetes Care* 21:2135–2139, 1998
13. Buckingham RE, Al-Barazanji KA, Toseland CD, *et al*: Peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, protects against nephropathy and pancreatic islet abnormalities in Zucker fatty rats. *Diabetes* 47:1326–1334, 1998
14. Jiang C, Ting AT, Seed B: PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* 391:82–86, 1998
15. Ricote M, Li AC, Willson TM, *et al*: The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 391:79–82, 1998
16. Zheng F, Fornoni A, Elliot SJ, *et al*: Upregulation of type I collagen by TGF-beta in mesangial cells is blocked by PPARgamma activation. *Am J Physiol Renal Physiol* 282:F639–F648, 2002
17. Ma LJ, Marcantoni C, Linton MF, *et al*: Peroxisome proliferator-activated receptor-gamma agonist troglitazone protects against nondiabetic glomerulosclerosis in rats. *Kidney Int* 59:1899–1910, 2001
18. Poynter ME, Daynes RA: Peroxisome proliferator-activated receptor alpha activation modulates cellular redox status, represses nuclear factor-kappaB signaling, and reduces inflammatory cytokine production in aging. *J Biol Chem* 273:32833–32841, 1998
19. Ruan H, Pownall HJ, Lodish HF: Troglitazone antagonizes TNF-alpha-induced reprogramming of adipocyte gene expression by inhibiting the transcriptional regulatory functions of NF-kB. *J Biol Chem* 278:28181–28192, 2003
20. Guan Y, Zhang Y, Davis L, Breyer MD: Expression of peroxisome proliferator-activated receptors in urinary tract of rabbits and humans. *Am J Physiol* 273:F1013–F1022, 1997
21. Guan Y, Breyer MD: Peroxisome proliferator-activated receptors (PPARs): Novel therapeutic targets in renal disease. *Kidney Int* 60:14–30, 2001
22. Baoutina A, Dean RT, Jessup W: Alpha-tocopherol supplementation of macrophages does not influence their ability to oxidize LDL. *J Lipid Res* 39:114–130, 1998
23. Wang Y, Chen J, Chen L, *et al*: Induction of monocyte chemoattractant protein-1 in proximal tubule cells by urinary protein. *J Am Soc Nephrol* 8:1537–1545, 1997
24. Zoja C, Donadelli R, Colleoni S, *et al*: Protein overload stimulates RANTES production by proximal tubular cells depending on NF-kappa B activation. *Kidney Int* 53:1608–1615, 1998
25. Tang S, Leung JC, Abe K, *et al*: Albumin stimulates interleukin-8 expression in proximal tubular epithelial cells in vitro and in vivo. *J Clin Invest* 111:515–527, 2003
26. Lee EM, Pollock CA, Drumm K, *et al*: Effects of pathophysiological concentrations of albumin on NHE3 activity and cell proliferation in primary cultures of human proximal tubule cells. *Am J Physiol Renal Physiol* 285:F748–F757, 2003
27. Niemeyer NV, Janney LM: Thiazolidinedione-induced edema. *Pharmacotherapy* 22:924–929, 2002
28. Ruan XZ, Moorhead JF, Fernando R, *et al*: PPAR agonists protect mesangial cells from interleukin 1beta-induced intracellular lipid accumulation by activating the ABCA1 cholesterol efflux pathway. *J Am Soc Nephrol* 14:593–600, 2003
29. Chinetti G, Griglio S, Antonucci M, *et al*: Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages. *J Biol Chem* 273:25573–25580, 1998
30. Ping D, Jones PL, Boss JM: TNF regulates the in vivo occupancy of both distal and proximal regulatory regions of the MCP-1/JE gene. *Immunity* 4:455–469, 1996